

### Claims

1. (Currently Amended) One or more computer-readable media comprising computer-executable instructions for performing a method to calculate concentration of a substance in a test sample, the method comprising:

after a second derivative is calculated for a standard sigmoid curve, finding a first point on the standard sigmoid curve via the second derivative of the standard sigmoid curve and designating the first point as a first bound;

finding a second point on the standard sigmoid curve via the second derivative of the standard sigmoid curve and designating the second point as a second bound;

determining a usable portion of the standard sigmoid curve as a plurality of points on the standard sigmoid curve between the first bound and the second bound;

for at least one observation of a metric for the test sample, determining whether the at least one observation is within the ~~finding where on a~~ usable portion of ~~[[a]]the~~ standard sigmoid curve ~~the observation lies, wherein a first endpoint and an other endpoint of the usable portion of the standard sigmoid curve are determined via a second derivative of the standard sigmoid curve, and the usable portion of the standard sigmoid curve comprises a range of a plurality of points between the first endpoint and the other endpoint;~~ and

~~based on a location of the observation on the standard sigmoid curve,~~ responsive to determining that the at least one observation is within the usable portion, calculating a concentration of the substance based on a location of the observation on the standard sigmoid curve.

2. (Currently Amended) The one or more computer-readable media of claim 1 wherein the standard sigmoid curve is represented via a four-parameter formula.

3. (Original) The one or more computer-readable media of claim 1 wherein the

standard sigmoid curve represents a sigmoid curve fit to a plurality of observations taken of a reference sample having a known concentration of the substance.

4. (Original) The one or more computer-readable media of claim 1 further comprising computer-executable instructions for performing the following:

determining for at least one observation of a metric for the test sample whether the observation is above a threshold value, wherein the threshold value is determined via a first derivative of the standard sigmoid curve; and

indicating whether the observation is above the threshold value.

5. (Original) The one or more computer-readable media of claim 1 wherein: the observation indicates optical density for the test sample.

6. (Original) The one or more computer-readable media of claim 5 wherein: the concentration indicates an amount of antibody in the test sample.

7. (Original) The one or more computer-readable media of claim 6 wherein: the concentration indicates an amount of anti-PA IgG in the test sample.

8. (Currently Amended) One or more computer-readable media comprising computer-executable instructions for performing a method to calculate concentration of a substance in a test sample, the method comprising:

for a plurality of observations of a metric for the test sample, fitting a test sigmoid curve to the observations;

**determining a usable portion of a standard curve, wherein the determining comprises performing (a)-(c):**

**(a) finding a first endpoint of the usable portion via a second derivative of the standard curve;**

**(b) finding a second endpoint of the usable portion via the second derivative of the standard curve; and**

**(c) determining the usable portion of the standard curve as a portion of the standard curve between the first endpoint and the second endpoint;**

**discarding one or more of the observations that are outside the usable portion of the standard curve; and**

calculating a concentration of the substance in the test sample via the test sigmoid curve and ~~[[a]]the~~ usable portion of ~~[[a]]the~~ standard curve, ~~wherein the usable portion of the standard sigmoid curve comprises a range of a plurality of points, wherein a first edge and a second edge of the range are determined via a second derivative of the standard sigmoid curve.~~

9. (Original) The one or more computer-readable media of claim 8 further comprising computer-executable instructions for performing the following:  
indicating the concentration of the substance.

10. (Original) The one or more computer-readable media of claim 8 further comprising computer-executable instructions for performing the following:  
displaying the concentration of the substance.

11. (Currently Amended) One or more computer-readable media comprising computer-executable instructions for performing a method to calculate concentration of a substance in a test sample, the method comprising:

finding a usable portion of a sigmoid curve, **wherein the finding comprises calculating a second derivative of the sigmoid curve and finding a first point and a second point on the sigmoid curve using the second derivative,** wherein ~~first and second endpoints of~~ the usable portion of the sigmoid curve **comprises a plurality of points on the sigmoid curve between the first point and the second point** ~~are determined via a second~~

~~derivative of the sigmoid curve, and the usable portion of the sigmoid curve comprises a range of a plurality of points between the first and second endpoints; and~~

calculating a concentration of the substance in the test sample via the usable portion of the sigmoid curve.

12. (Currently Amended) One or more computer-readable media comprising computer-executable instructions for performing a method comprising:

for a plurality of dilutions of a test sample, receiving respective measurements of optical density indicating concentration of live cells within the dilutions;

designating a first point and a second point on a sigmoid curve using a second derivative of the sigmoid curve;

defining a plurality of points on the sigmoid curve between the first point and the second point as a usable portion of the sigmoid curve;

discarding one or more of the measurements that are outside the usable portion of the sigmoid curve;

via the measurements, calculating a concentration of anti-PA IgG for the test sample via ~~[[a]] the~~ usable portion of a sigmoid curve representing concentrations of live cells within dilutions of a reference sample having a known quantity of anti-PA IgG, wherein the sigmoid curve is represented via a four-parameter logistic technique, ~~and wherein a usable portion of the sigmoid curve comprises a range of a plurality of points between two bounds determined via a second derivative of the sigmoid curve;~~ and

indicating the concentration of anti-PA IgG for the test sample.

13. (Currently Amended) A computer-implemented method of calculating concentration of a substance in a test sample having an unknown concentration of the substance, the method comprising:

determining a usable portion of a sigmoid curve fit to data points representing observations of a reference sample having a known concentration of the substance by

**calculating second derivative values for the sigmoid curve and selecting a first point and a second point on the sigmoid curve using the second derivative values**, wherein the usable portion of the sigmoid curve comprises a range of a plurality of points **between the first point and the second point** ~~representing a range of observational values~~;

**based on the usable portion of the sigmoid curve, selecting from a plurality of observations of the test sample, a subset of observations of the test sample within the usable portion of the sigmoid curve**; and

calculating the concentration of the substance in the test sample based on ~~[[a]]~~ **the** subset of observations of the test sample, ~~wherein the subset is within the range of observational values represented by the usable portion of the sigmoid curve.~~

14. (Currently Amended) The method of claim 13 further comprising:

excluding at least one ~~excluded observation~~ **of the plurality of observations** of the test sample responsive to determining the **at least one of the plurality of observations** ~~excluded observation~~ is outside the usable portion of the sigmoid curve.

15. (Canceled)

16. (Currently Amended) The method of claim 13 wherein ~~determining a usable portion of the sigmoid curve comprises designating a portion between~~ **the first point and the second point correspond to** a minimum and a maximum of ~~[[a]]~~ **the** second derivative ~~values for the sigmoid curve as the usable portion of the sigmoid curve.~~

17. (Currently Amended) The method of claim 13 wherein a **third** point on the sigmoid curve relating to a threshold for a first derivative of the sigmoid curve is used as a lower threshold to indicate presence of the substance.

18. (Currently Amended) A computer-implemented method of determining the

concentration of antibody in a blood serum sample, the method comprising:

receiving a measurement indicative of concentration of live cells in a test sample,  
wherein the test sample is generated by adding the serum to cells and a toxin neutralized by the  
antibody;

**determining a usable portion of a standard sigmoid curve representing  
observations taken of a sample having a known concentration of antibody, wherein the  
determining comprises performing (a)-(c):**

**(a) calculating second derivative values for the standard sigmoid curve;**

**(b) selecting a first point and a second point on the standard sigmoid curve using  
the second derivative values; and**

**(c) determining the usable portion of the standard sigmoid curve as a plurality of  
points on the standard sigmoid curve between the first point and the second point;**

determining whether the measurement falls within ~~[[a]]the~~ usable portion of ~~[[a]] the~~  
standard sigmoid curve ~~representing observations taken of a sample having a known~~  
~~concentration of antibody, wherein the usable portion of the standard sigmoid curve~~  
~~comprises a range of a plurality of points representing a range of observations; and~~

~~responsive to determining when~~ the measurement falls within the usable portion,  
calculating a concentration via the **measurement and the usable portion of the** standard  
sigmoid curve.

19. (Currently Amended) One or more computer-readable media having computer-  
executable instructions for performing a method of determining the concentration of antibody  
in a blood serum sample, the method comprising:

receiving a measurement indicative of concentration of live cells in a test sample,  
wherein the test sample is generated by adding the serum to cells and a toxin neutralized by the  
antibody;

**determining a usable portion of a standard sigmoid curve representing  
observations taken of a sample having a known concentration of antibody, wherein the**

**determining comprises performing (a)-(c):**

**(a) calculating second derivative values for the standard sigmoid curve;**

**(b) selecting a first point and a second point on the standard sigmoid curve using the second derivative values; and**

**(c) determining the usable portion of the standard sigmoid curve as a plurality of points on the standard sigmoid curve between the first point and the second point;**

determining whether the measurement falls within ~~[[a]]~~the usable portion of ~~[[a]]~~ the standard sigmoid curve ~~representing observations taken of a sample having a known concentration of antibody, wherein the usable portion of the standard sigmoid curve comprises a range of a plurality of points representing a range of observations; and responsive to determining when~~ the measurement falls within the usable portion, calculating a concentration via the measurement and the usable portion of the standard sigmoid curve.

20. (Original) The method of claim 18 wherein results for plural test samples for plural dilutions of an original test sample are included in the calculating.

21. (Original) The method of claim 18 wherein concentration of live cells is indicated by optical density of the test sample.

22. (Canceled)

23. (Original) The method of claim 18 wherein the antibody is anti-PA IgG.

24. (Currently Amended) The method of claim 18 further comprising:  
discarding the measurement at least one observation having a concentration of live cells when the measurement is outside the usable portion of the standard sigmoid curve.

25. (Canceled)

26. (Currently Amended) A software system encoded on one or more computer-readable media, the software system comprising:

a representation of a characteristic sigmoid curve;

means for designating a usable portion of the characteristic sigmoid curve by calculating a second derivative for the characteristic sigmoid curve and selecting a first point and a second point on the characteristic sigmoid curve using the second derivative, wherein first and last endpoints of the usable portion of the characteristic sigmoid curve are determined via a second derivative and wherein the usable portion comprises a range of a plurality of points between the first point and the second point ~~the first and last endpoints;~~

means for receiving at least one observation of a test sample;

means for determining whether the observation of the test sample is within the usable portion of the characteristic sigmoid curve; and

means for calculating a concentration for the observation responsive to determining that the observation is within the usable portion of the characteristic sigmoid curve.

27. (Canceled)

28. (Canceled)

29. (Original) The software system of claim 26 further comprising:

means for rejecting an observation responsive to determining that the observation is outside the usable portion of the characteristic sigmoid curve.

30. (Currently Amended) One or more computer-readable media comprising computer-executable instructions for performing a method to indicate presence of a substance



in a test sample, the method comprising:

for at least one observation of a metric for the test sample, determining whether the observation is higher than a threshold value, wherein the threshold value is determined via a first derivative of a standard sigmoid curve; **and**

responsive to determining the observation is higher than the threshold value, indicating presence of the substance;

**calculating a second derivative of the standard sigmoid curve;**

**selecting a first point and a second point on the standard sigmoid curve using the second derivative;**

**defining a plurality of points on the standard sigmoid curve between the first point and the second point as a usable portion of the standard sigmoid curve; and**

**calculating a concentration of the substance in the test sample when the at least one observation falls within the usable portion of the standard sigmoid curve.**

31. (New) The one or more computer-readable media of claim 1 wherein the finding of the first point and the second point on the standard sigmoid curve is performed using a local maximum of the second derivative, a local minimum of the second derivative, an inflection point of the second derivative, or a combination thereof.

32. (New) The one or more computer-readable media of claim 1 wherein the first point corresponds to a local maximum of the second derivative and the second point corresponds to a local minimum of the second derivative.

33. (New) The one or more computer-readable media of claim 1 wherein the method further comprises:

when the at least one observation is not within the usable portion, indicating that the at least one observation is not used in the calculating of the concentration of the substance.

34. (New) The one or more computer-readable media of claim 1 wherein:  
the usable portion corresponds to a range of measurement values; and  
the determining of whether the observation is within the usable portion of the standard sigmoid curve comprises determining whether a measurement value associated with the at least one observation is within the range of measurement values.

35. (New) The one or more computer-readable media of claim 8 wherein the method further comprises:

selecting from the plurality of observations a subset of observations that falls within the usable portion of the standard curve, wherein the calculating of the concentration is performed using the subset of observations and the usable portion of the standard curve.